

PHYSIOLOGICAL OBSERVATIONS ON STARVATION AND DESICCATION OF THE SNAIL AUSTRALORBIS GLABRATUS

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It has been shown that planorbid snails, all of which are aquatic pulmonates, can withstand desiccation rather well both in nature and under laboratory conditions (Precht, 1939; Olivier, 1956a, 1956b; Olivier and Barbosa, 1955, 1956). The physiology of desiccating planorbids has, however, received scant attention. Magalhães Neto (1954) observed that five specimens of *Australorbis glabratus* showed a considerably decreased rate of oxygen consumption during desiccation at an unspecified relative humidity. Desiccating aquatic snails retract into their shells; they are unable to feed and hence come under conditions of starvation. Since starvation decreases the rate of oxygen consumption (von Brand, Nolan and Mann, 1948), and since the anatomical relationship of a retracted snail to the source of oxygen is quite different from that of an active one, the following questions arise: Is the reduction in oxygen consumption due mainly to starvation, to difficulties in securing sufficient oxygen, or to desiccation proper? These and related questions are discussed in the present paper.

MATERIAL AND METHODS

Laboratory-reared albino *Australorbis glabratus*, derived from a normally pigmented Venezuelan strain, were used in preference to pigmented specimens because the heart-beat could easily be seen through the shell. This was important, not only because a study of the heart rate under desiccation was interesting in itself, but also in order to establish whether a snail was alive or dead. The usual procedure of placing a desiccated snail in water to observe whether it resumes its normal activities could not be employed because in most of our experiments repeated measurements with the same specimens were required, or because a chemical determination had to be made on desiccated specimens. Most of the snails that appeared dead, as judged by cessation of the heart-beat, were tested further by placing them in water. Of about 200 such snails, only three revived, indicating that our death criterion was reasonably accurate.

All snails initially weighed between 180 and 350 mg. and had fed *ad libitum*. They were freed of excess moisture as described previously (Newton and von Brand, 1955) and weighed to the nearest mg. During starvation, snails (minimum of 28 per series) were kept individually in numbered beakers filled with dechlorinated tap water. They were shifted daily to fresh beakers during the first week of starvation and thereafter twice weekly. During desiccation, snails (minimum of

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33 per series) were put into individual dry beakers and these were kept at the same temperature ($27 \pm 1^\circ \text{C.}$) as the starvation series in desiccators over water or over saturated salt solutions giving a desired relative humidity. The solutions employed gave the following relative humidities, as determined by an Aminco electric hygrometer.

H_2O giving 95 to 97 per cent relative humidity, average 96 per cent
 ZnSO_4 giving 83 to 87 per cent relative humidity, average 85 per cent
 NaCl giving 72 to 76 per cent relative humidity, average 74 per cent
 NaBr giving 57 per cent relative humidity, average 57 per cent
 CaCl_2 giving 28 to 31 per cent relative humidity, average 30 per cent
 LiCl giving 13 to 17 per cent relative humidity, average 15 per cent

When survival, weight, heart rate, and rate of oxygen consumption were determined, all snails alive on a given day were used. At the end of the determinations they were returned to the desiccators, or the water-containing beakers, respectively. These snails were used repeatedly until the last specimen died. When chemical determinations were done, only the specimens to be analyzed on a given day were used, while the others remained undisturbed until required for analysis.

The heart rate was determined by counting the heart-beats for one minute under a dissecting microscope.

The conventional Warburg technique was used for the oxygen consumption. The vessels contained 2 ml. of dechlorinated tap water in the starvation series and in the experiments designed to give the pre-desiccation rate. In the desiccation experiments the snails were put into the main compartment of the vessel without water. In these cases the side arms of all vessels, including thermobarometer, contained 0.3 ml. of the same salt solution that was present in the desiccators where the snails had been kept, thus maintaining approximately the same relative humidity. In all cases the temperature was 30°C.

Polysaccharides were determined according to von Brand's (1936) micro-modification of Pflüger's method. Total lipids were determined by heating the crushed snail with 30 per cent NaOH in a boiling water bath, acidifying the solution with 7 per cent H_2SO_4 , extracting the solution three times with ether, washing the combined ether fractions with distilled water, evaporating the ether, and weighing the lipids on a microbalance after drying at 80°C. For lactic acid² and volatile acids the methods of Barker and Summerson (1941) and Bueding (1949) were used, respectively.

All measurements were done on numbered individual snails, with the exception of the volatile acid determinations, where two snails were used. All values are expressed on the basis of the initial, pre-experimental weight of the snails.

RESULTS

1. *Survival.* Figure 1 shows the survival groups of starving snails at various relative humidities and in water. It is obvious that time of survival decreased with decreasing humidity. When the 50 per cent death times are plotted logarithmically against relative humidity (lower part of Fig. 1), no straight line

² We are indebted to Mr. C. Elwood Claggett for carrying out the lactic acid determinations.

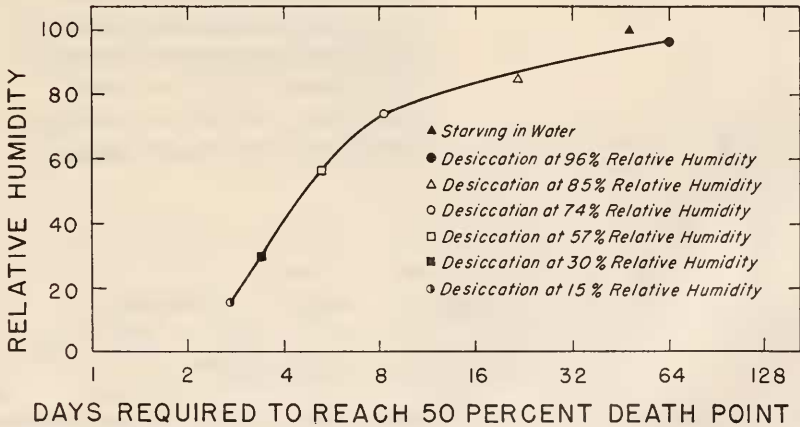
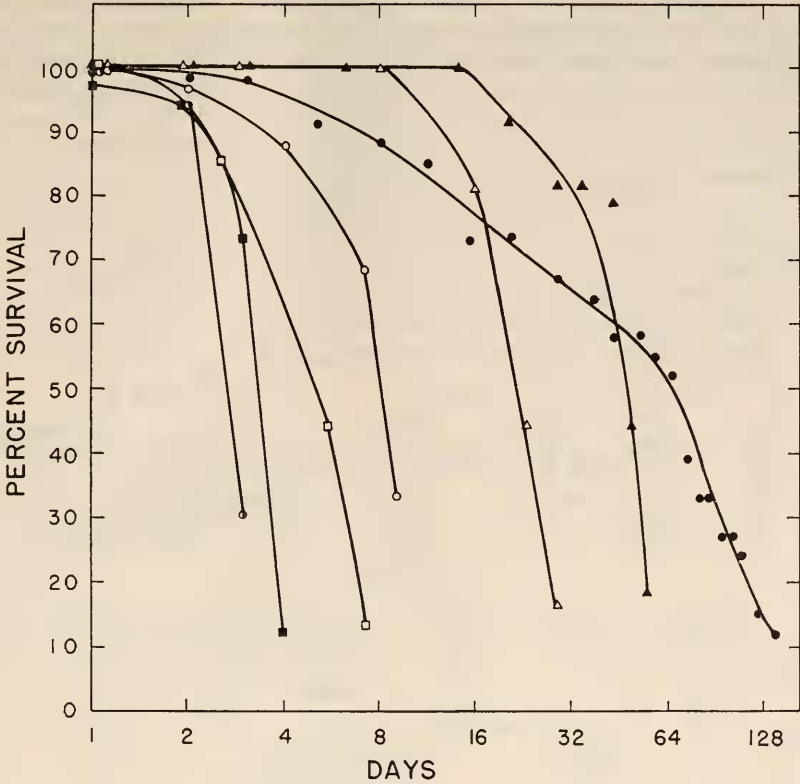


FIGURE 1. Survival of *Australorbis glabratus* starving in water or desiccating at various relative humidities. At the beginning each desiccating series consisted of 33 specimens, the water-starvation series of 28 specimens. The same groups of snails yielded the results shown in Figures 2, 3 and 4.

results. The shape of the curve indicates that no fixed relationship between time of survival and relative humidity exists, but that decreasing humidity leads progressively to an ever more accelerated death rate. Survival during starvation in water was only about half as long as during desiccation over water (96 per cent relative

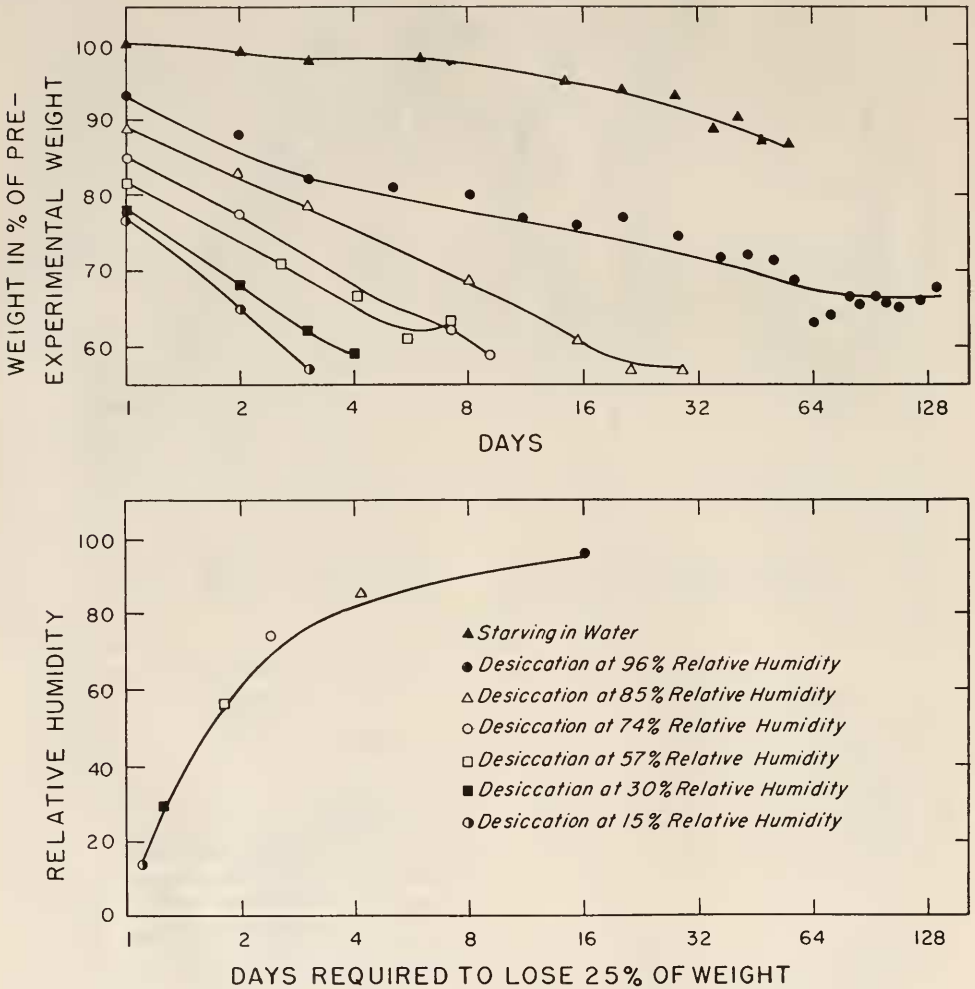


FIGURE 2. Weight relationships of *Australorbis glabratus* starving in water or desiccating at various relative humidities.

humidity). However, the 50 per cent death points of these two series were closer together. This is due to the fact that the first deaths due solely to starvation occurred later than those from the combined influences of starvation and desiccation.

2. *Weight loss.* Thirty fed control snails averaged 40.8 per cent dry substance. The shells of 30 other controls, after the soft parts had been removed according to Nolan and von Brand's (1954) procedure averaged 31.6 per cent of the total

weight. The dry weight of the soft tissues was thus 9.2 per cent of the total body weight. Since the shell contains practically no water, the total water found can be ascribed to the soft tissues. Their initial over-all hydration is then calculated as 87 per cent (Table III).

A total weight loss of 13 per cent was observed in snails starving to death in water. It was hence greater than the total dry weight of the soft tissues initially present and it must be concluded that it was due in part to a loss of water, perhaps corresponding to the hydration water of the metabolized organic material.

The weight loss of desiccating snails (Fig. 2) was much more pronounced than that of snails starving in water and was clearly dependent on the relative humidity. The lower part of Figure 2 indicates that the relationship between humidity and weight loss is very similar to that described above for survival. Snails desiccating and starving at 96 per cent relative humidity metabolize during 128 days approxi-

TABLE I

Desiccation of Australorbis glabratus at 85 per cent relative humidity. The figures are per cent of the pre-desiccation values; the figure following the \pm sign is the standard error of the mean

Days of desiccation	Heart rate		Weight		Rate of O ₂ consumption		Per cent survival
	A	B	A	B	A	B	
1	98 \pm 2.6	98 \pm 4.2	89 \pm 0.5	90 \pm 0.7	49 \pm 2.6	47 \pm 4.5	100
2	100 \pm 4.2	107 \pm 5.2	83 \pm 0.3	85 \pm 1.7	46 \pm 2.2	47 \pm 4.4	100
3	95 \pm 3.0	93 \pm 4.8	79 \pm 0.7	81 \pm 0.9	33 \pm 2.7	30 \pm 3.0	100
8	105 \pm 3.7	98 \pm 3.7	69 \pm 1.0	71 \pm 1.1	27 \pm 2.1	20 \pm 2.4	100
15	93 \pm 4.6	95 \pm 3.7	61 \pm 1.1	64 \pm 1.7	28 \pm 2.5	30 \pm 3.6	81
22	81 \pm 9.9	81 \pm 9.9	57 \pm 1.6	57 \pm 1.6	10 \pm 2.0	10 \pm 2.0	44
28	81 \pm 2.4		57 \pm 3.7		19 \pm 5.0		16

The initial number of snails was 33.

A = Values of all snails alive at specified day.

B = Values of all snails surviving on day 22.

mately 50 to 60 per cent of their organic material (corresponding to 4-5 per cent of the pre-experimental live body weight, see Discussion). Since in the humidity range of 15 to 85 per cent practically all snails had died by day 20, their loss of organic material must have been much smaller, and no appreciable error can be introduced if the entire weight loss is here ascribed to loss of water. At these humidities the weight had declined terminally to 60 per cent of the initial, corresponding to a loss of $40/59.2 =$ approximately 70 per cent of the initial water.

At 96 per cent humidity, on the other hand, survival was much longer and the weight at death was higher, amounting to 65 to 70 per cent of the initial weight. As mentioned above, about 4 to 5 per cent weight loss must in this case be ascribed to metabolized organic material. It is therefore clear that at this high humidity the last animals died before being desiccated to quite the same degree as in the other desiccation series. It is probable that in this case starvation was a contributing factor to death.

It should be realized that the figures summarized in Figure 2 (the following applies also to the data presented in Figs. 3 and 4) are averages for all snails

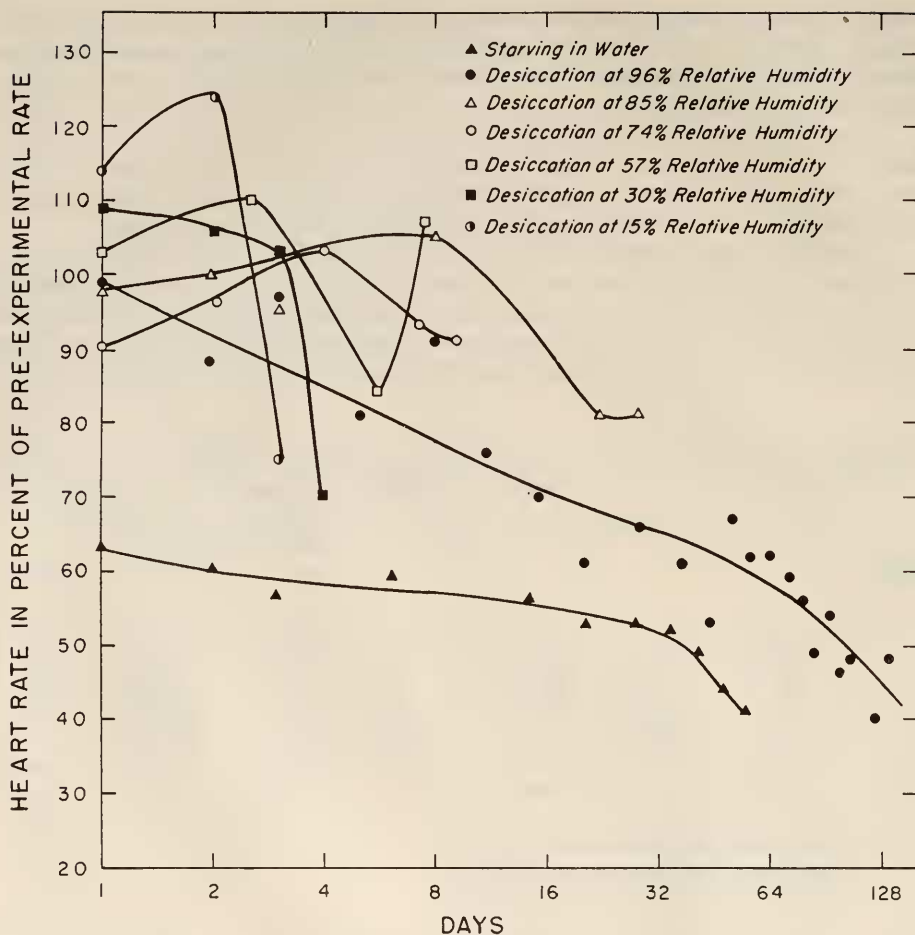


FIGURE 3. Heart rates of *Australorbis glabratus* starving in water or desiccating at various relative humidities.

alive at a specified day. They therefore represent the average changes occurring in a population. Some irregularities in the curves, especially noticeable towards the end of an experiment, are due to the summation of experimental errors and the slightly variable behavior of the individual snails. The curves do not change materially, however, if the data are restricted to snails which are still alive on a day nearing the end of a given experiment, provided the number is sufficient to give a valid average. This is illustrated for one of our series in Table I for weight and other criteria studied.

3. *Heart rate.* The heart rate of snails (Fig. 3) starving in water slowed precipitously to 63 per cent of the original rate during the first 24 hours of starvation. During the remainder of the starvation period the heart rate declined slowly further, the final value being about 40 per cent of the initial one. In snails desiccating at 96 per cent relative humidity there was no decline during the first 24 hours,

but thereafter the heart rate became progressively slower, reaching about the same end-point as in snails starving in water.

A different situation prevailed in snails desiccating at all lower humidities. Within the first few days, there was a period when the heart-beat increased in frequency above the pre-experimental value, this period being followed by one of more or less precipitous decline. While the heart-beat was generally full and regular in snails in water and in air at 96 per cent relative humidity, many irregularities were observed at lower humidities, such as partial contractions of the heart, or

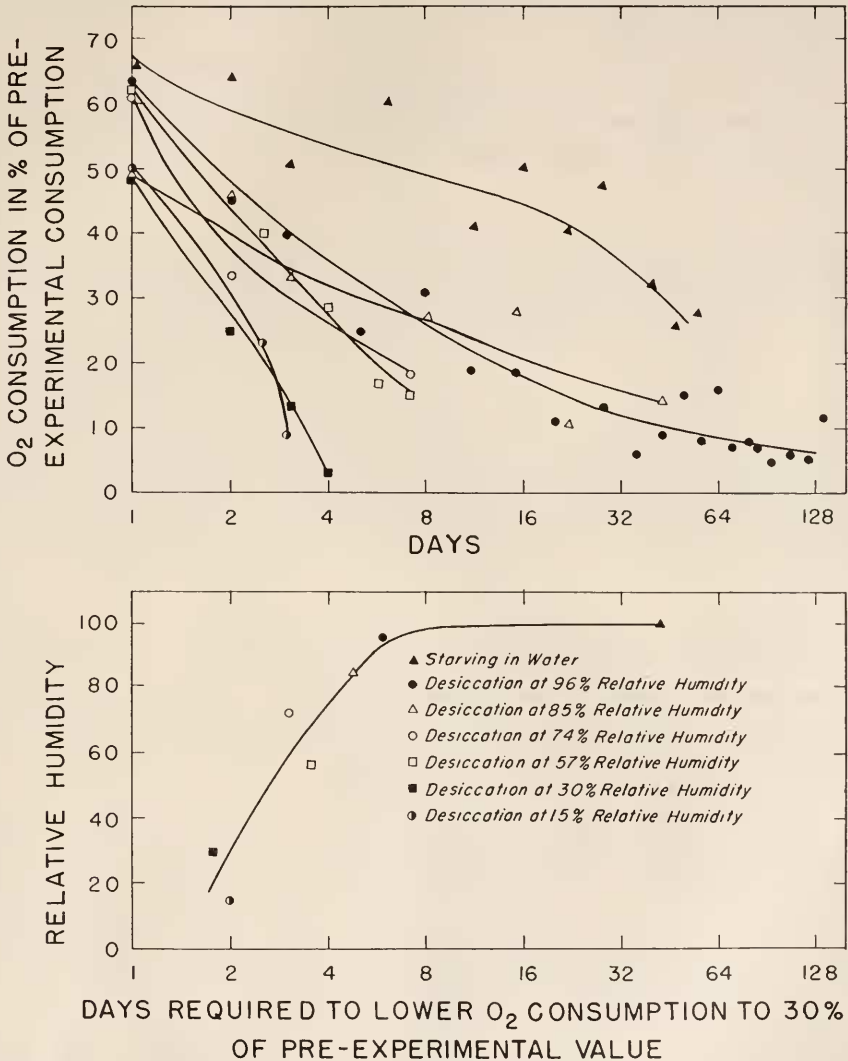


FIGURE 4. Oxygen consumption of *Australorbis glabratus* starving in water or desiccating at various relative humidities.

cessation of the pulsations for a few seconds followed by a period of very rapid contractions. It should be noted that at 57 per cent humidity the last value raises the curve (Fig. 3.) This artifact is due to abnormally high rates in all three surviving snails.

Heart rate apparently had no direct relation to survival under our conditions. For instance, in our series of 33 snails desiccating at 96 per cent relative humidity, 7 snails with initial heart rates of 33 to 37 beats per minute survived an average of 48 days. The other extreme was represented by 5 snails with initial rates of 50 to 63 beats/minute and an average survival of 80 days. After 128 days of desiccation, 5 snails still survived; their average initial rate was 42 beats/minute with 37 and 53 as extremes.

4. *Oxygen consumption.* The rate of oxygen consumption (Fig. 4) declined in all series more or less rapidly, but the decline was slower in the water-starvation than in the desiccation series. The daily variations were more pronounced in the former than in the latter, possibly due to the motility of the snails starving in water

TABLE II

Chemical determinations on Australorbis glabratus starving in water or desiccating at 96 per cent relative humidity. All values have been calculated on the basis of the pre-starvation or pre-desiccation live weight of the snails. The figure following the \pm sign is the standard error of the mean, the figure in parenthesis indicates the number of determinations

Days	Per cent lipids		Per cent polysaccharides		μ Lactic acid	μ Volatile acid*
	Desiccation	Starvation	Desiccation	Starvation	Desiccation	Desiccation
0	0.76 \pm 0.023 (24)	0.65 \pm 0.036 (24)	1.03 \pm 0.10 (23)	1.29 \pm 0.16 (22)	140 \pm 27.8 (12)	53 \pm 28.1 (6)
10	0.58 \pm 0.019 (24)	0.43 \pm 0.026 (24)	0.77 \pm 0.07 (22)	1.04 \pm 0.18 (24)	117 \pm 28.1 (10)	11 \pm 5.4 (6)
20	0.54 \pm 0.016 (20)	0.38 \pm 0.011 (19)	0.69 \pm 0.07 (24)	0.69 \pm 0.14 (21)	35 \pm 7.9 (12)	18 \pm 12.8 (6)
30	0.52 \pm 0.019 (19)	0.36 \pm 0.011 (19)	0.56 \pm 0.08 (20)	0.59 \pm 0.12 (22)	0.0 (11)	23 \pm 3.9 (3)

* The volatile acids are expressed as acetic acid, since this is the predominant volatile fatty acid (Mehlman and von Brand, 1951).

as contrasted with the immobility of the desiccating specimens. In the starvation series the final rate was about 30 per cent of the pre-experimental one, while in the desiccation series the endpoint varied from about 20 to well below 10 per cent of the initial value. The rapidity of decline (lower half of Fig. 4) showed a rough correlation with degree of humidity, but it was not so close as that shown between humidity and survival or humidity and weight loss.

A rough inverse correlation probably exists between survival and initial rate of oxygen consumption. Taking the series of snails desiccating at 96% relative humidity, as example, six snails had initial rates varying between 205 and 277 mm.³ O₂/gm./hr. with an average survival of 51 days. In four snails the rate varied initially between 82 and 108 mm.³ O₂/gm./hr. and their average survival was 103 days. The five snails surviving 128 days desiccation had an initial rate of 143 mm.³ O₂/gm./hr., with 106 and 171 mm.³ as extremes.

5. *Chemical determinations.* Chemical determinations were performed only during the first 30 days on animals starving in water and desiccating at 96 per cent relative humidity since snails desiccating at lower humidities died too early. In view of the variability in storage of reserve substances, it was essential to limit

periods of exposure to experimental conditions to those tolerated by all or at least the great majority of specimens employed, since otherwise a possible differential death rate between snails with high and low initial reserves would make valid conclusions impossible.

The data summarized in Table II show that starving and desiccating snails use appreciable amounts of both polysaccharide and lipids, the consumption being more pronounced during the first 10 days than after prolonged exposure to experimental conditions. Snails starving in water used little more of these reserve substances than did the desiccating specimens. In desiccating snails the lactic acid initially present in the tissues disappeared completely within 30 days, while the volatile acid content decreased only slightly.

DISCUSSION

The laboratory strain of *Australorbis glabratus* used in the present studies withstood desiccation fairly well. As was expected, the snails retracted into their shells. They were not capable of forming a true epiphragm which in many other species is an efficient mechanism for preventing excessive loss of water, nor did they produce complete mucus membranes across the shell aperture, an auxiliary mechanism frequently employed (Gebhardt-Dunkel, 1953). Partial mucus membranes were observed occasionally, but they did not seem to change the rate of evaporation materially.

Marked reduction in the rate of oxygen consumption with time was characteristic at all humidities studied and a loose inverse correlation with humidity existed. This reduction was not due solely, and in the series at low humidities not even primarily, to starvation. Snails starving in water maintained a higher rate of oxygen consumption than the desiccating specimens; they must therefore have used their reserve substances at a faster rate. It would then seem that the amount of reserve substances available to the desiccating animals would have sufficed to maintain an equal rate of oxygen consumption if starvation alone were involved. An altered anatomical relationship to the source of oxygen can also be eliminated as the cause of this reduction. If difficulties in securing oxygen played a significant role, a partial shift to anaerobiosis would have been expected. It should be noted in this connection that a partial shift to anaerobiosis can readily be induced in *Australorbis* by exposure to low concentrations of pentachlorophenol (Weinbach and Nolan, 1956) and that a considerable increase in lactic acid content has been reported from aestivating *Pila* (Meenakshi, 1956). In our desiccating specimens, on the contrary, the lactic acid present initially disappeared completely and the volatile acids diminished. There is little doubt that *Australorbis*, at least during desiccation at high humidity, maintained a purely aerobic metabolism despite the deep retraction into the shell.

The lung of a contracted snail is probably largely compressed and it is problematical whether it plays a large role in the gaseous exchanges. Diffusion through the tissues exposed to the air within the shell may have been sufficient. It should be kept in mind that conditions are quite different when a snail retracts into its shell in water. During desiccation the tissues are in direct contact with atmospheric air where the absolute amounts of oxygen are much higher than in water and where diffusion is incomparably more rapid than if the whorl is filled with water. It is

therefore most likely that the reduction in oxygen consumption was largely a consequence of desiccation proper, although in the longer-lasting series starvation may well have been a contributing factor.

That loss of water *per se* influences snails can be deduced also from our heart-beat observations. Starvation in water led to a reduction in rate, and a similar reduction, though slower to appear, was evident in snails desiccating at 96 per cent relative humidity. At lower humidities, on the contrary, the period of decline was preceded by one of increased rate and many irregularities in heart action were observed. While the final water loss was not very different, it was more gradual in the 96 per cent series and a difference in over-all tissue hydration was probably present even towards the end of the experiments (see below). On the whole, the impression was gained that at 96 per cent humidity the heart had an opportunity to adapt itself to changed conditions, while this did not occur during the shorter periods involved at lower humidities. It is probable that at the lower humidities increased concentration of organic and inorganic materials accumulating in the blood may have put a strain on the heart. It was not directly demonstrated because *Australorbis* is for technical reasons not suitable for such experiments, but Arvanitaki and Cardot (1932) had found previously a salt concentration of 0.080 N in *Helix pisana* collected immediately after a rain and 0.147 N seven days afterwards.³

In humidities of 85 per cent and below, the last snails died when they had lost about 70 per cent of their original water.⁴ While this loss is very large,⁵ the over-all tissue hydration does not decline to the same extent, because the remaining water hydrates the tissues of an animal whose weight has declined. The general relations between over-all tissue hydration and total water loss are shown in Figure 5. This figure is drawn on the assumption that the organic material remains unchanged, and is therefore valid only in cases of very rapid desiccation. In experiments of long duration, such as at 96 per cent relative humidity, a considerable percentage of tissue is lost. While no exact data could be secured, a final loss of 50 to 60 per cent appears possible (see below). If this loss is taken into account, the over-all tissue hydration was about the same at the beginning as at the end of the desiccation period (Table III). Even if this should be literally true (and no such claim is made), the physiological state of the desiccating snail probably differs from that of snails kept in water. For instance, any loss of water, whether accompanied by a loss of tissue or not, should result in an increased percentage of inorganic material

³ It is probably unwarranted, however, to generalize: Pusswald (1948) reported that the blood of the slugs *Arion* and *Limax* lost 84.5 and 92.0 per cent of their initial water, respectively, when the water loss of the entire body was 60 per cent. In *Limax* the percentage of water content of the blood had at this point declined from 97.6 per cent to 77.3 per cent. In snails with external shells the water loss of the blood seems to be less pronounced. Gebhardt-Dunkel (1953) studied five species of terrestrial snails and found a decline in the water content of the blood from initial values ranging in the various species from 97.7 to 98.1 per cent to final values varying between 88.4 and 88.8 per cent shortly before death from desiccation.

⁴ Actually, the water loss is probably slightly higher since the water resulting from the oxidation of food reserves has not been taken into account in this calculation.

⁵ This resistance to loss of water is not unique. Roots (1956) states that the earthworms *Allolobophora chlorotica* and *Lumbricus terrestris* survive losses of body water of 75 and 70 per cent, respectively. Other invertebrates are more sensitive. According to Biancamaria (1955), the crayfish *Potamon edulis* dies after having lost 15 to 23 per cent of the original water. For older data on resistance to desiccation, see Hall (1922).

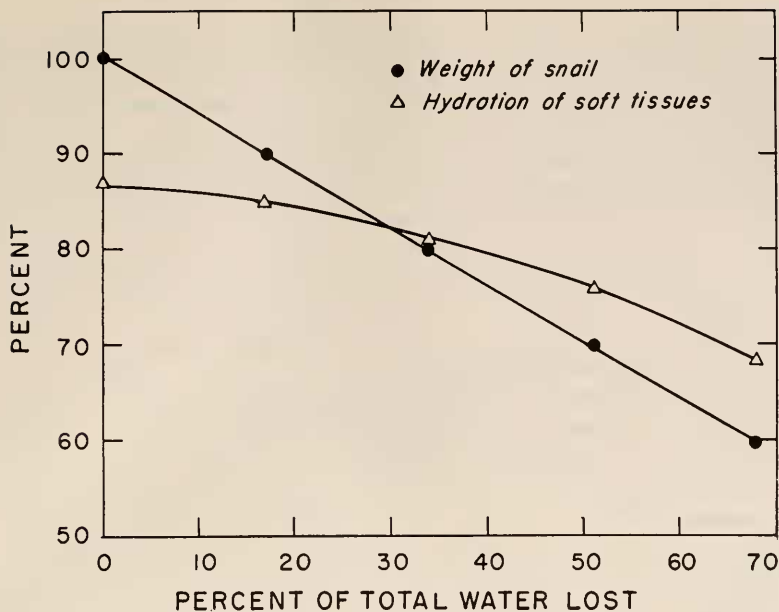


FIGURE 5. Theoretical relation between weight (water loss) and over-all hydration of soft tissues in desiccating *Australorbis glabratus*. Initial water content 87 per cent.

in the remaining tissues, unless the snail is capable of incorporating the excess into the shell, a point that was not studied. Computations from data presented by Buck and Keister (1949, Fig. 7) show that in flies, also, considerable water loss may occur without decrease in over-all tissue hydration. This suggests that the phenomenon may be widespread.

The endogenous foodstuffs during desiccation were studied only at 96 per cent relative humidity where a consumption of polysaccharides, lipids, lactic and volatile acids was found. Since there was no indication of a partial shift to anaerobiosis, total oxidation can be assumed and the oxygen required for it can be calculated. It is also possible to calculate approximately the total oxygen consumed during the desiccation period by graphic integration of the rates determined at the intervals shown in Figure 4. As Figure 6 indicates, the above substances account only for

TABLE III

Calculated over-all hydration of the soft tissues of *Australorbis glabratus* when 50 per cent of the soft tissues disappear during desiccation at 96 per cent relative humidity and the final total weight of the desiccated snail is 67 per cent of the pre-desiccation value, as was found

	Total weight, mg.	Shell weight, mg.	Weight of soft tissues, mg.	Weight of water, mg.	Per cent water in complex soft tissues + water
Pre-desiccation	100	31.6	9.2	59.2	87
Post-desiccation	67	31.6	4.6	30.8	87

a relatively small fraction of the total oxygen consumption, both during desiccation and during starvation in water. In conformity with other starving organisms, it may be assumed that proteins were the main substrate. A calculation of the total oxygen consumed by snails desiccating at 96 per cent relative humidity for 128 days gives approximately 56 ml. oxygen per one gram original weight. About 9 ml. are accounted for by the oxidation of polysaccharides and lipids during the first 30 days (the additional amount for lactic and volatile acids is negligible). Since these reserves were largely depleted at the end of this period, 47 ml. of oxygen can

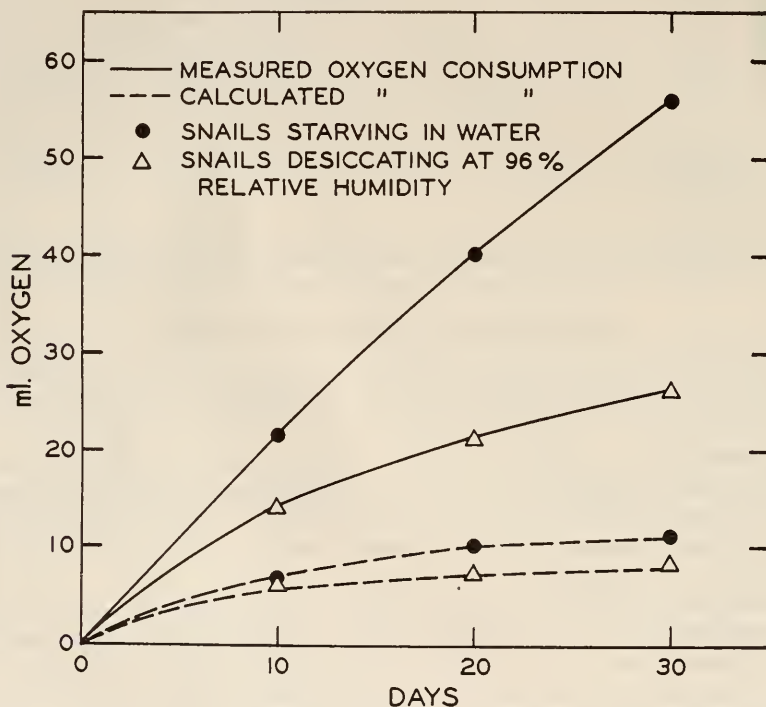


FIGURE 6. Comparison of oxygen consumption calculated from polysaccharide and lipid consumption, assuming total oxidation, and measured oxygen consumption (total oxygen consumption obtained by graphic evaluation of determinations done at specified intervals; see text). The values have been calculated for one gram pre-starvation or pre-desiccation weight.

tentatively be linked with protein consumption, permitting the oxidation of approximately 48 mg. of protein. Since a one-gram snail (initial weight) contains, on an average, 92 mg. of dried soft tissue, the calculated tissue loss would be roughly 50 to 60 per cent. This figure appears possible, since even higher organisms can lose more than 50 per cent of their weight during starvation, *e.g.*, a dog discussed by Pütter (1911) decreased in weight from 19.65 kg. to 9.17 kg., yet recovered upon feeding. Other examples are planarians, which decrease so markedly in size during starvation that their weight loss must be far larger than 50 per cent (Stoppenbrinck, 1905; Berninger, 1911).

SUMMARY

1. Decreasing humidity leads to a progressively more rapid decline of survival time, body weight and rate of oxygen consumption. Snails starving in air of high humidity survive longer than snails starving in water, but their final weight is lower.

2. The heart rate of snails starving in water or desiccating at 96 per cent relative humidity decreases. At all lower humidities a transitory phase of increased heart rate and many irregularities in heart action occurs.

3. During starvation in water and during desiccation, polysaccharide and lipid stores become depleted. Lactic acid disappears completely from the tissues during desiccation and volatile acids diminish.

4. It is concluded that the decrease in oxygen consumption is largely due to desiccation proper but that at high humidity starvation is a contributing factor.

5. Snails desiccating at high humidity have a purely aerobic metabolism. The relationship between the oxygen required for oxidation of polysaccharides and lipids and the total oxygen consumed indicates that protein may be the main substrate during prolonged periods of starvation in water or of desiccation.

6. The percentage of total body water lost and the percentage of water in the tissues do not decrease at the same rate during desiccation, tissue hydration declining at a slower rate. If marked tissue losses occur during long periods of desiccation, the over-all tissue hydration may remain unchanged even if the total water loss is very pronounced.

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